Stimulatory Effect of Bradykinin (BK) on Glucagon Secretion From the Perfused Rat Pancreas: Involvement of BK_2 Receptors

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The purpose of the study is to investigate the direct effect of bradykinin (BK), a potent vasoactive nonapeptide, on glucagon secretion from the perfused rat pancreas. BK (0.1, 1, and 10 μmol/L) increased glucagon secretion in a concentration-dependent manner. HOE 140, a BK_2 receptor antagonist (0.01, 0.1, and 1 nmol/L), prevented the stimulatory effect of BK on glucagon secretion in a concentration-dependent manner. In contrast, des-Arg^9,Leu^8–BK, a BK_1 receptor antagonist (1 nmol/L), failed to antagonize the effect of BK. Thus, BK stimulates glucagon secretion from the perfused rat pancreas by activating BK_2 receptors, but not BK_1 receptors.

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THE AUTACOID BRADYKININ (BK) is a potent vasodilator nonapeptide released from kininogen by the enzymatic action of the protease kallikrein.[1] Kinins, including BK, are involved in multiple biologic processes including induction of hypotension and an increase in vascular permeability,[2] production of pain and inflammation,[3] contraction of smooth muscle,[4] and vasodilatation due to an increase in the generation of nitric oxide.[5] At the cellular level, BK increases cell proliferation,[6] promotes the transport of chloride[4] and glucose,[7] and decreases blood glucose.[8]

Kinins stimulate the release of renin from the kidney, vasopressin from the posterior pituitary gland, catecholamines from the adrenal medulla,[4] and growth hormone and prolactin from the anterior pituitary gland.[9,10] Kinins also stimulate the release of platelet-activating factors and prostaglandins,[11] leukotrienes and substance P,[12] acetylcholine,[13] and cytokines.[14] Two subtypes of BK receptors, BK_1 and BK_2, have been identified: BK_1 receptors mediate the acute inflammatory response, whereas BK_2 receptors mediate most of the effects of BK.[15]

The pancreas is one of the richest sources of tissue kallikrein,[4] which is found in the acini and islets, with most of it located in the acini.[16] We found that BK increased insulin secretion in a concentration-dependent manner in the perfused rat pancreas.[17] The effects of BK were mediated by BK_2 receptors, since HOE 140 (D-Arg[Hyp^3,Thi^5,D-Tic^7,Oic^8]BK), a BK_2 receptor antagonist, reduced basal insulin secretion, abolished the effect of BK on insulin secretion, and attenuated glucose-induced insulin secretion.[18] BK-induced insulin secretion was glucose-dependent since it failed to stimulate insulin secretion in the absence of extracellular glucose. In addition, glucose potentiated BK-induced insulin secretion in a glucose concentration–dependent manner.[18] We also found that 1 μmol/L BK increased insulin and glucagon secretion while decreasing somatostatin secretion from the perfused rat pancreas.[19]

The present study was designed to investigate the dose-response of BK on glucagon secretion from the perfused rat pancreas and to characterize the BK receptor subtype which mediates its effect on glucagon secretion from the pancreas.

MATERIALS AND METHODS

Male Sprague-Dawley rats (400 to 500 g) were used. The rats were anesthetized with pentobarbital sodium (60 mg/kg intraperitoneally) and were maintained at 37°C on a hot plate during the experiment. Pancreatic perfusion was performed as previously described.[17] Briefly, after cannulation of the celiac artery, the rat pancreas was immediately perfused with Krebs-Ringer bicarbonate (KRB) solution supplemented with 20 mmol/L HEPES, 1.4 mmol/L glucose, 1% dextran, and 0.2% bovine serum albumin as a basal medium. The KRB was maintained at pH 7.4 and continuously aerated with 95% O_2-5% CO_2. The rats were killed by the induction of pneumothorax immediately following cannulation of the portal vein and the beginning of the flow.

Experimental Design

The first 20 minutes of perfusion was considered an equilibration period. Subsequently, the effluent fluid was collected every minute from the cannula in the portal vein. The flow rate was set at 1 mL/min. In experiment 1, after a baseline period of 18 minutes, the medium containing BK (0.1, 1, or 10 μmol/L) was administered for 10 minutes, followed by a washout period during which the basal medium was administered for 10 minutes. In experiment 2, after a baseline period of 8 minutes, the pancreas was pretreated for 10 minutes with a medium containing HOE 140, a BK_2 receptor antagonist (0.01, 0.1, or 1 nmol/L), or des-Arg^9,Leu^8–BK, a BK_1 receptor antagonist (1 nmol/L). This was followed by the medium containing BK (1 μmol/L) and HOE 140 or des-Arg^9,Leu^8–BK for 10 minutes, and the basal medium for another 10 minutes for the washout period. Control experiments were performed following the same protocol except that KRB was used in place of the agents. In all experiments, arginine (1 mmol/L) was administered at the end of the experiment for 5 minutes to stimulate glucagon secretion to confirm that the tissue retained the normal secretory capacity under the experimental condition. The effluent fluids were kept at 4°C and analyzed within 6 hours for glucagon using a radioimmunoassay as previously described.[20]

Test Agents

BK and des-Arg^9,Leu^8–BK were purchased from Sigma Chemical (St Louis, MO). HOE 140 was donated by Hoechst-Roussel Pharmaceuticals (Somerville, NJ). All agents were dissolved in distilled water to make stock solutions. These solutions were further diluted with KRB (basal medium) to attain appropriate concentrations. Glucagon standard was donated by Eli Lilly Laboratories (Indianapolis, IN). Glucagon antibody was donated by Dr Joseph Dunbar of Wayne State University

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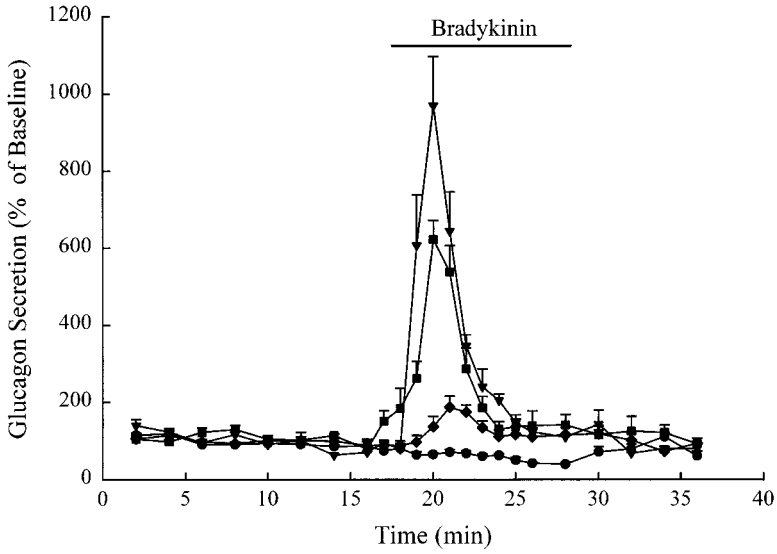

Fig 1. Effect of BK on glucagon secretion from the perfused rat pancreas. In these experiments, a 20-minute equilibration period preceded time 0. After a baseline period of 18 minutes, BK was administered for 10 minutes, followed by 10 minutes of the washout period. Values are the mean ± SE (n = 4). Basal control (●); BK 0.1 (◆), 1 (■), and 10 (▼) μmol/L. Range of baseline glucagon concentration of effluents, 307-792 pg/mL.

(Detroit, MI). ^{125}I-glucagon was purchased from Linco Research (St. Charles, MO).

Statistical Analysis

The effluent concentration of glucagon is expressed as a percentage (mean ± SE) of the baseline level (mean of last 5 baseline values). The area under the curve for the BK treatment period of 10 minutes was calculated using the Transforms and Regressions program (Sigma Plot 4.0; SPSS, Chicago, IL). The data were analyzed using the SAS Proc General Linear Means procedure (SAS Institute, Cary, NC). Individual mean comparisons were calculated using the F test. The significance level was set at *P* less than .05.

RESULTS

Effects of BK on Glucagon Secretion

Glucagon secretion remained constant during the first 32 minutes in the control group receiving 1.4 mmol/L glucose only. Administration of 1 mmol/L arginine in the control group increased glucagon secretion by 7.6 times over the baseline level at the end of the experiment (data not shown). BK (0.1, 1, or 10 μmol/L) increased glucagon secretion and reached a peak of 0.9, 5.1, and 8.7 times, respectively, over the baseline level (Fig 1). BK increased glucagon secretion in a biphasic pattern—a peak followed by a sustained phase. The onset time of glucagon secretion was less than 1 minute and reached the maximum within 3 minutes of BK administration. The sustained glucagon secretion induced by BK (0.1, 1, or 10 μmol/L) was 0.5 to 2.0 times the baseline level. The effluent glucagon concentration returned to the baseline during the washout period (no BK) and increased to 6 to 20 times the baseline value upon administration of 1 mmol/L arginine (data not shown). By calculating the areas under the curve for BK treatments and comparing them with the corresponding area of the control group, BK (0.1, 1, and 10 μmol/L) significantly increased glucagon secretion in a concentration-dependent manner (Fig 2). After BK administration, the flow rate remained constant (1 mL/min) during the perfusion.

Effects of BK Antagonists on BK-Induced Increase in Glucagon Secretion

BK (1 μmol/L) alone increased glucagon secretion by 5.1 times over the baseline level (Fig 2). Since this concentration of BK caused an optimal increase in glucagon secretion, we chose it for the antagonism study. By calculating the areas under the curve for BK antagonist treatments and comparing them with the corresponding area of the BK-only group, pretreatment of the pancreas with HOE 140, a BK_2 receptor antagonist (0.01, 0.1, or 1 nmol/L) for 10 minutes, antagonized the effect of BK on glucagon secretion in a concentration-dependent manner. HOE 140 at the highest concentration studied (1 nmol/L) abolished BK-induced glucagon secretion. In contrast, the BK_1 receptor antagonist des-Arg^9,Leu^8–BK (1 nmol/L) failed to antagonize BK-induced glucagon secretion (Figs 3 and 4). None

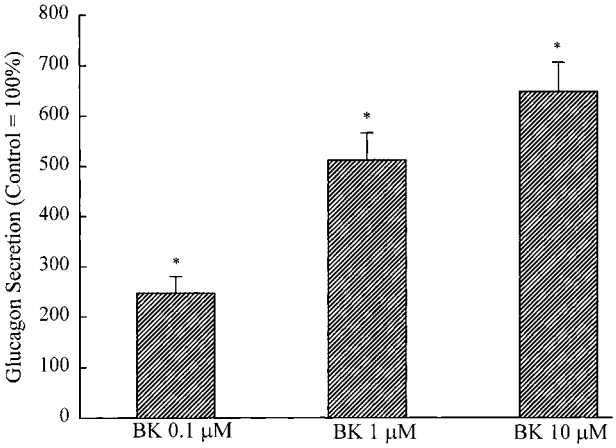

Fig 2. Effect of BK on glucagon secretion from the perfused rat pancreas. Values are the mean ± SE (n = 4), obtained by calculating the area under the 10-minute glucagon secretion curve for BK treatments, and are expressed as a percentage of the control group. *$P < .05$ *v* control.

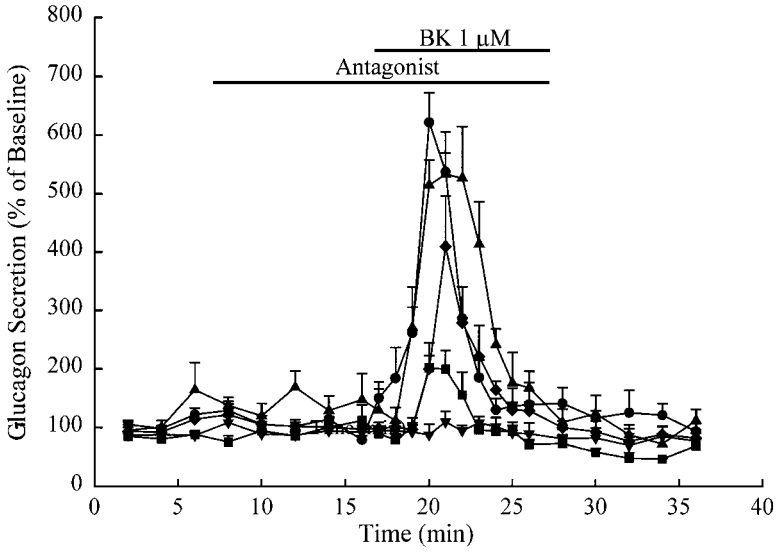

Fig 3. Effects of BK receptor antagonists on BK-induced increase in glucagon secretion from the perfused rat pancreas. In these experiments, a 20-minute equilibration period preceded time 0. After a baseline period of 8 minutes, HOE 140 or des-Arg^9,Leu^8–BK, was administered for 10 minutes. This was followed by the medium containing BK (1 μmol/L) and HOE 140 or des-Arg^9,Leu^8–BK for 10 minutes, and the basal medium for another 10 minutes for the washout period. BK 1 μmol/L (●); des-Arg^9,Leu^8–BK 1 nmol/L + BK 1 μmol/L (▲); HOE 140 0.01 nmol/L + BK 1 μmol/L (◆); HOE 140 0.1 nmol/L + BK 1 μmol/L (■); HOE 140 1 nmol/L + BK 1 μmol/L (▼). Range of baseline glucagon concentration of effluents, 186-672 pg/mL.

of these antagonists alone at the concentrations studied had an effect on glucagon secretion (Fig 3).

DISCUSSION

In the present study, BK (0.01 to 10 μmol/L) induced glucagon secretion from the perfused rat pancreas in a concentration-dependent manner. These findings indicate that BK may physiologically increase glucagon secretion, because 2 of the 3 BK concentrations used in this study (0.1 and 1 μmol/L) were lower than that in the rat pancreas (3 μmol/L).[19] The pancreas is one of the richest sources of tissue kallikrein,[4] which should promote the formation of kinins in this organ.

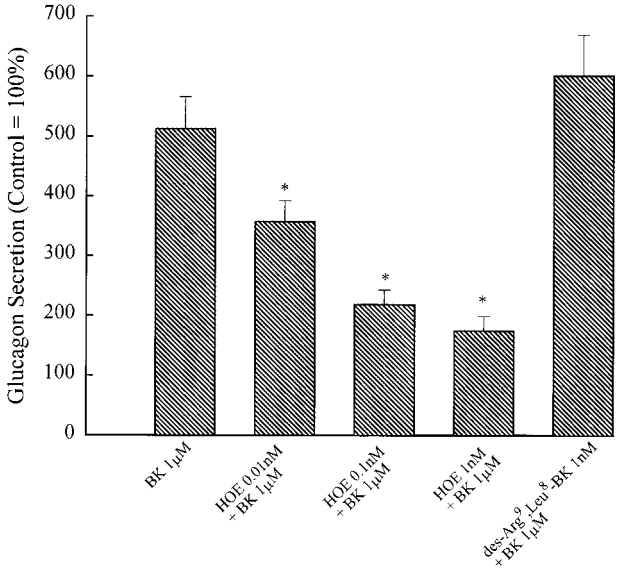

Fig 4. Effects of BK receptor antagonists on BK-induced increase in glucagon secretion from the perfused rat pancreas. Values are the mean ± SE (n = 4), obtained by calculating the area under the 10-minute glucagon secretion curve for antagonist + BK treatments, and are expressed as a percentage of the control group. *$P < .05$ *v* BK-only group.

Most of the kinin actions are mediated by BK_2 receptors.[9] BK_1 receptors seem to be absent normally and are present only in pathologic states such as inflammation.[4] Thus, it is not surprising that the stimulatory effect of BK on glucagon secretion is mediated by BK_2 receptors, since HOE 140 (0.01 to 1 nmol/L), a specific BK_2 receptor antagonist, antagonized the effect of BK on glucagon secretion in a concentration-dependent manner. HOE 140 at 1 nmol/L completely abolished BK-induced glucagon secretion. In contrast, des-Arg^9,Leu^8–BK (1 nmol/L), a BK_1 receptor antagonist, failed to antagonize the effect of BK on glucagon secretion. HOE 140 (0.1 and 1 μmol/L) antagonized BK-induced insulin secretion from the perfused rat pancreas[19] and clonal β-cell line RINm5F,[21] respectively, whereas des-Arg^9,Leu^8–BK (1 μmol/L) did not affect BK-induced insulin secretion from the clonal β-cell line RINm5F.[21] Therefore, BK increases insulin and glucagon secretion by activating BK_2 receptors in the rat pancreas.

In the present study, we used very small concentrations of HOE 140 (0.01 to 1 nmol/L) to antagonize the effect of BK, of which 1 nmol/L completely abolished BK-induced glucagon secretion. Our results support the notion that HOE 140 is a potent BK_2 receptor antagonist. HOE 140 alone (≤1 nmol/L) did not affect baseline glucagon secretion, but HOE 140 at 100 nmol/L decreased baseline insulin secretion.[17] It is not known whether a high concentration of HOE 140 (100 nmol/L) will also inhibit baseline glucagon secretion.

In the presence of 1.4 mmol/L glucose, BK at 1 μmol/L caused a peak level of 5.1 times over the baseline level, and this increase was still noticeable during the 10 minutes of BK administration. However, in the presence of 6 mmol/L glucose, the same concentration of BK (1 μmol/L) caused only a 70% increase in glucagon secretion, and this increase subsided within 2 minutes of BK administration.[19] This difference is attributable to the fact that a low glucose level inhibits insulin secretion, which may alleviate the negative effect of insulin on glucagon secretion.[22,23]

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